

# *APOBEC3 Host Factors Modulate Viral Production and Infectivity of HIV-2*

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### *Review and Aims*

Several studies indicate that susceptibility to host factors could explain pathogenic differences between HIV-2 and HIV-1. Deaminases of APOBEC3 (A3) family, particularly A3G, have antiviral activity against HIV-1 which is suppressed by the viral protein Vif. Little is known concerning HIV-2 inhibition by the different members of the APOBEC3 family.

Probably HIV-2 and HIV-1 have differences in the threshold requirements for the same cellular factor or in the involvement of different factors to compensate for Vif1 and Vif2 functions<sup>2,3</sup> considering that :

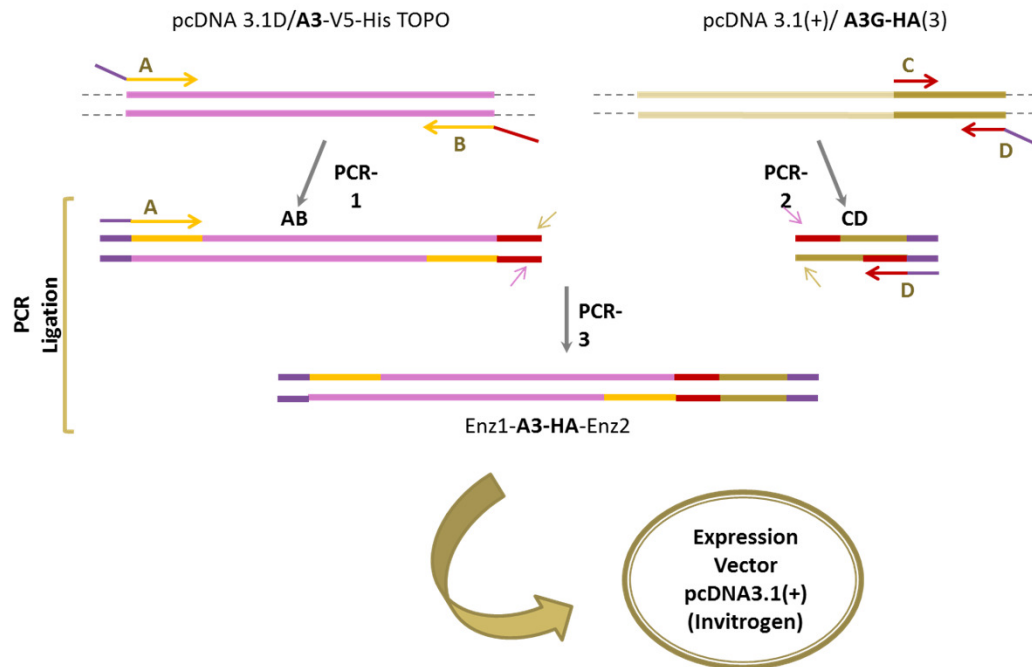
- Vif2 and Vif1 share only 30% of identity
- Vif2-induced degradation of A3 proteins is different from the one previously observed by Vif1.<sup>1</sup>
- HIV-2 and HIV-1 vif defective viruses produced in different T-cell lines showed differential replication and capacity for productive infection

**Our aim is to characterize the effect of several A3 proteins in HIV-2 production and infectivity, in order to understand the differences between HIV-1 and HIV-2 and to characterize specific determinants of HIV-2 pathogenesis.**

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### *Methods*

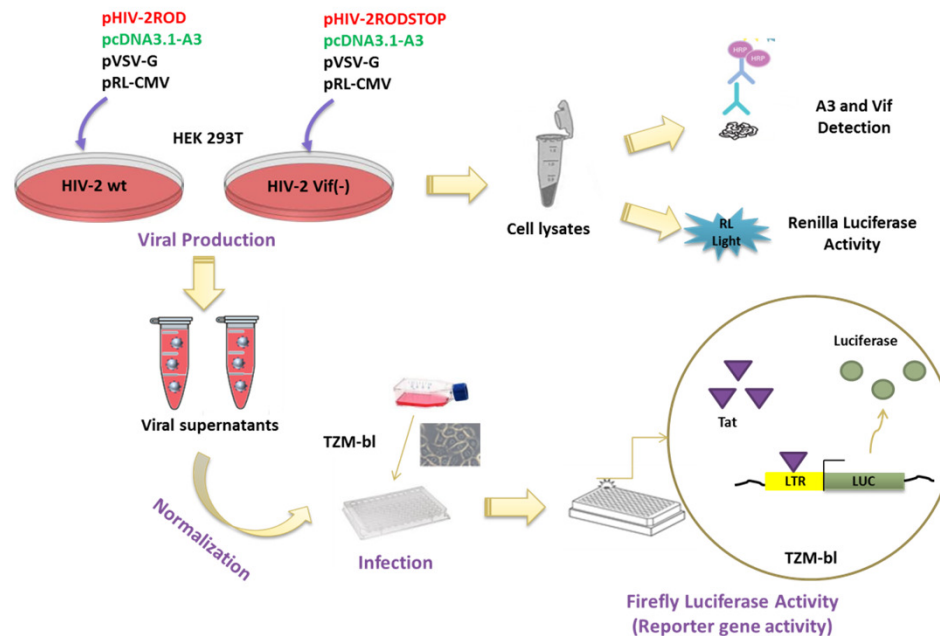
**STEP1:** Several A3 genes were cloned in the same expression vector system, expressing A3 in fusion with 3 HA-tags in C-terminal, using PCR ligation strategy :



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**STEP 2:** Viral production in HEK293T cells, protein detection in cell lysates and infection of TZM-bl cells with HIV-2 and HIV-1 wild type (wt) and Vif defective (Vif-) virions, produced in the absence or presence of different A3 proteins.

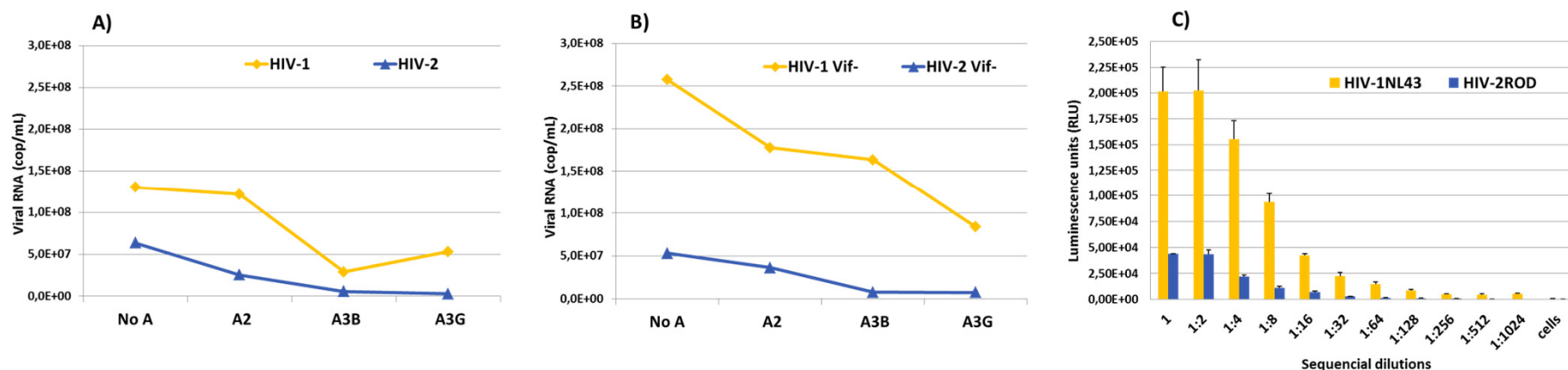


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### Results

Viral production of HIV-1 is higher than HIV-2 production in 293T cells transfected with the same amount of DNA.

Infectivity detection system used has a higher discriminatory power for HIV-1 infections.

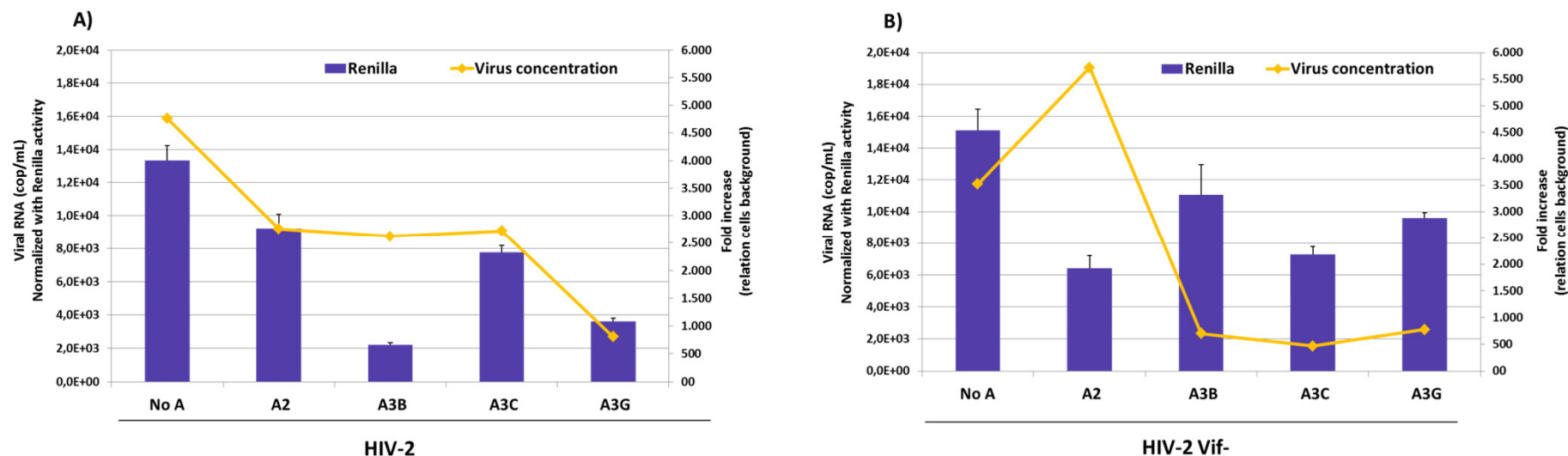


**Fig. 1 Differences in production levels of HIV-2 and HIV-1.** A), B) 293T cells were transfected with HIV<sub>WT</sub> and HIV Vif- molecular clones, in the presence or absence of several A3 expression vectors in ratio of 1:1 HIV:A3 (1 $\mu$ g of each DNA). Supernatant viral RNA levels was determined by RT-PCR in Virology Laboratory of Egas Moniz Hospital using specific primers for LTR region of HIV-2 and ENV region of HIV-1. Results from one representative assay. C) TZM-bl infectivity values using different dilutions of HIV-2 and HIV-1<sub>WT</sub>

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### *Results*

Viral production is affected by the presence of A3 proteins and by the efficiency of transfection. In the presence of A3 proteins, HIV-2Vif- production decreases drastically.

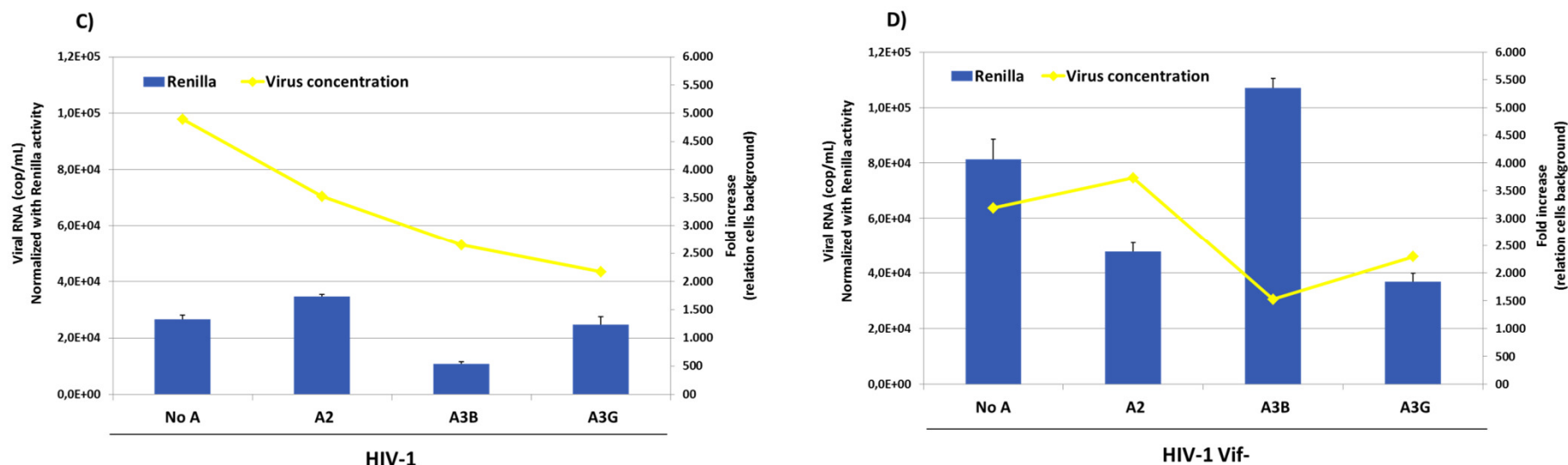


**Fig. 2 A3 proteins inhibit viral production of HIV-2 (A) and HIV-2 vif-(B) .** pRL.CMV (Renilla expression vector) it was used as a transfection efficiency control of 293T producing cells . Values of Renilla activity are presented as n fold in relation to cell background (secondary axis). Produced viral levels (copies/mL) were normalized with values of transfection efficiency control determined in the producing cell lysates for each condition.

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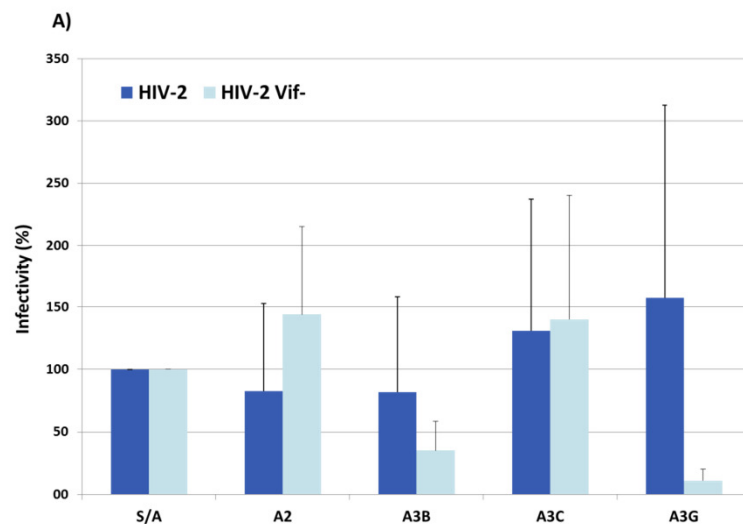


**Fig. A3 proteins inhibit viral production of HIV-1 (C) and HIV-1 vif- (D)** pRL.CMV (Renilla expression vector) it was used as a transfection efficiency control of 293T producing cells. Values of Renilla activity are presented as n fold in relation to cell background (secondary axis). Produced viral levels (cop/mL) were normalized with values of transfection efficiency control determined in the producing cell lysates for each condition. *Porque mudou o tipo de letra?*

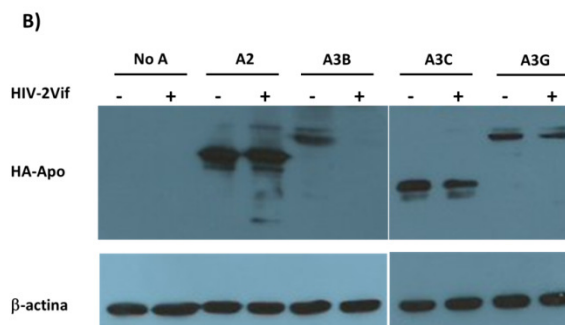
## APOBEC3 Host Factors Modulate Viral Production and Infectivity of HIV-2

### Results

A3B and A3G inhibit HIV-2 Vif- infectivity and they are strongly neutralized by Vif2.



Vif2-induced degradation of A3B and A3G in producing cells lysates



**Fig. 3 A)** Viral infectivity of HIV-2wt and HIV-2Vif- produced in the presence of different APOBEC. After virus normalization, A3 antiviral effects were evaluated by one cycle infectivity assay in TZM-bl cells. Values are presented as % of infectivity relative to viruses produced in the absence of A3, defined as 100% (empty vector was used as control). Columns and error bars represent average  $\pm$  SD, respectively (n=2); **B) Sensitivity of A3 proteins to Vif2.** Representative Western blot of A3 proteins in virus-producing cells lysates.



## *Conclusions*

- In transfected 293T cells HIV-1 production is higher than HIV-2 production.
- Viral production is affected by the presence of A3 and by the efficiency of transfection.
- This effect is more pronounced in HIV-2Vif- productions.
- HIV-2 infectivity is strongly inhibited by A3B and A3G and this antiviral effect is efficiently suppressed by Vif2 protein.

In the near future, we will compare HIV-2 and HIV-1 infectivity using viral stocks produced in the presence of lower amounts of A3, to avoid the effect on viral production.

## *References*

- [1] Smith, J.L., et al., (2014) Journal of Virology, 88(17), p. 9893-9908
- [2] Reddy, T.R., et al., (1995) Journal of Virology, 69(6), p. 3549-3553.
- [3] Ribeiro, A.C. et al., (2005) Journal of Virology, 79, p.823-833.

## *Acknowledgements*

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